



UNIVERSITI PUTRA MALAYSIA

**EVALUATION OF MALAYSIAN ISOLATES OF TRICHODERMA
HARZIANUM RIFAI AND GLIOCLADIUM VIRENS MILLER,
GIDDENS AND FOSTER FOR THE BIOLOGICAL CONTROL OF
SCLEROTIUM FOOT ROT OF CHILLI**

JINANTANA JOMDUANG

FH 1995 5

EVALUATION OF MALAYSIAN ISOLATES OF *TRICHODERMA HARZIANUM* RIFAI
AND *GLIOCLADIUM VIRENS* MILLER, GIDDENS AND FOSTER FOR THE
BIOLOGICAL CONTROL OF SCLEROTIUM FOOT ROT OF CHILLI

By

JINANTANA JOMDUANG

Dissertation Submitted in Fulfilment of
the Requirements for the Degree of Doctor of Philosophy
in the Faculty of Agriculture,
Universiti Pertanian Malaysia.

April 1995



To my mother and my husband
for their love which nourishes my inspiration

ACKNOWLEDGEMENTS

Deepest and sincere gratitude is expressed to Associate Professor Dr. Sariah Meon who has successively guided, supervised, encouraged and supported all the success of my academic work.

Heartful thanks are also expressed to Dr. Mohamad Zakaria Bin Hussin and Dr. Mohammad Md. Ali for their invaluable advice during conducting the research and preparing the final manuscript of dissertation.

Sincere appreciation is devoted to the Rajamangala Institute of Technology, Bangkok, Thailand and the International Maize and Wheat Improvement Center, Mexico D.F., Mexico, for the financial grants. Special appreciation is also extended to the International Mycological Institute, Kew, Surrey, England, for the fungal identification service.

Grateful acknowledges are extended to the staff members of the Department of Plant Protection, the vegetable experimental field, the Ladang Dua experimental field and the Electron Microscope Unit, Universiti Pertanian Malaysia, for their help and cooperation.

Special thanks are also extended to all friends for their kindly assistance in the glasshouse and field experiments.

Lastly, thank you very much to my elder sister, Ms. Jirawan Kongchit, for every support she gave me during the study.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	xi
LIST OF PLATES.....	xii
ABSTRACT.....	xvi
ABSTRAK.....	xx
CHAPTER	
I INTRODUCTION.....	1
II LITERATURE REVIEW.....	6
Sclerotium Foot Rot of Chilli.....	6
Economic Importance of <i>S.rolfsii</i>	7
Biology and Epidemiology of <i>S.rolfsii</i>	8
Control Strategies of Disease Caused by <i>S.rolfsii</i> with Particular Reference to Antagonistic Fungi.....	14
Occurrence and Distribution of <i>T. harzianum</i> and <i>G. virens</i>	20
Factors Affecting Growth of <i>T. harzianum</i> and <i>G. virens</i>	21
Survival and Proliferation of <i>T. harzianum</i> and <i>G. virens</i> in Soil and Plant Rhizosphere.....	23

	Page
III INFECTION MECHANISM OF <i>SCLEROTIUM ROLFSII</i> ON CHILLI.....	25
Introduction.....	25
Materials and Methods.....	27
Isolation and Identification of <i>S.rolfsii</i>	27
Infection Mechanism of <i>S. rolfsii</i> on Chilli Stem Segments.....	28
Infection Mechanism of <i>S. rolfsii</i> on Chilli Plants.....	30
Effect of Age of the Chilli Plants and Inoculum Density of <i>S. rolfsii</i> on Disease Incidence.....	31
Results.....	32
Isolation and Identification of <i>S.rolfsii</i>	32
Infection Mechanism of <i>S. rolfsii</i> on Chilli Stem Segments.....	32
Infection Mechanism of <i>S. rolfsii</i> on Chilli Plants.....	41
Effect of Age of the Chilli Plants and Inoculum Density of <i>S. rolfsii</i> on Disease Incidence.....	47
Discussion.....	50
IV <i>TRICHODERMA HARZIANUM</i> AND <i>GLIOCLADIUM VIRENS</i> AS ANTAGONISTS OF <i>SCLEROTIUM ROLFSII</i>	55
Introduction.....	55
Materials and Methods.....	56
Isolation and Identification of <i>T. harzianum</i> and <i>G. virens</i>	56

	Page
In Vitro Screening for <i>T. harzianum</i> and <i>G. virens</i> Isolates Antagonistic to <i>S. rolfsii</i>	58
Antagonism Mechanism of Selected <i>T. harzianum</i> and <i>G. virens</i> Isolates against <i>S. rolfsii</i>	60
Predisposing Factors Affecting Growth and Proliferation of Isolates of <i>T. harzianum</i> and <i>G. virens</i>	64
Inhibition Studies between <i>T. harzianum</i> and <i>G. virens</i>	66
Results.....	68
Isolation and Identification of <i>T. harzianum</i> and <i>G. virens</i>	68
In Vitro Screening for <i>T. harzianum</i> and <i>G. virens</i> Isolates Antagonistic to <i>S. rolfsii</i>	72
Antagonism Mechanism of <i>T. harzianum</i> IMI No. (353529) and <i>G. virens</i> (IMI No. 353523) against <i>S. rolfsii</i>	79
Predisposing Factors Affecting Growth and Proliferation of Isolates of <i>T. harzianum</i> and <i>G. virens</i>	90
Inhibition Studies between <i>T. harzianum</i> and <i>G. virens</i>	98
Discussion.....	98
V POTENTIAL OF <i>TRICHODERMA HARZIANUM</i> AND <i>GLIOCLADIUM VIRENS</i> AS BIOCONTROL AGENTS IN CONTROLLING SCLEROTIUM FOOT ROT OF CHILLI.....	108
Introduction.....	108
Materials and Methods.....	110
Shelf-life of Air-dried <i>T. harzianum</i> and <i>G. virens</i> Incorporated to Organic fertilizers.....	110

	Page
Efficacy of <i>T. harzianum</i> and <i>G. virens</i> in Controlling Sclerotium Foot Rot of Chilli under Glasshouse Condition.....	113
Efficacy of <i>T. harzianum</i> and <i>G. virens</i> in Controlling Sclerotium Foot Rot of Chilli in Small Scale Field Trial.....	117
Results.....	122
Shelf-life of Air-dried <i>T. harzianum</i> and <i>G. virens</i> Incorporated to Organic Fertilizers.....	122
Efficacy of <i>T. harzianum</i> and <i>G. virens</i> in Controlling Sclerotium Foot Rot of Chilli under Glasshouse Condition.....	125
Efficacy of <i>T. harzianum</i> and <i>G. virens</i> in Controlling Sclerotium Foot Rot of Chilli in Small Scale Field Trial.....	134
Discussion.....	141
VI GENERAL DISCUSSION AND CONCLUSION.....	147
BIBLIOGRAPHY.....	154
APPENDIX	
A Additional Tables.....	167
B Abstract of Papers Published.....	171
VITA.....	175



LIST OF TABLES

Table		Page
1	Percentages of Disease Incidence in the 2- and 4-month-old Chilli Plants Inoculated with Different Numbers of <i>S. rolfsii</i> -infested Wheat Seed.....	49
2	Isolates of <i>T. harzianum</i> and <i>G. virens</i> from Different Sources.....	68
3	Antagonistic Effects of <i>T. harzianum</i> and <i>G. virens</i> Isolates to <i>S. rolfsii</i> in Dual Culture.....	73
4	Antagonistic Effects of <i>T. harzianum</i> and <i>G. virens</i> Isolates to <i>S. rolfsii</i> in the Colony Degradation Test.....	76
5	Effect of Volatile Inhibitors Produced by <i>T. harzianum</i> and <i>G. virens</i> on Radial Growth of <i>S. rolfsii</i> on PDA.....	89
6	Effect of Non-volatile Inhibitors Produced by <i>T. harzianum</i> and <i>G. virens</i> on Radial Growth of <i>S. rolfsii</i> on PDA.....	90
7	Trade Name, Formulation and Components of the Three Commercially Available Organic Fertilizers Used in the Present Study.....	112
8	Populations of Air-dried <i>T. harzianum</i> , <i>G. virens</i> and <i>T. harzianum</i> + <i>G. virens</i> Cultures Incorporated into Three Organic Fertilizers and Stored at Room Temperature for 0-180 days.....	124
9	Number of Viable <i>S. rolfsii</i> Propagules in Soil around the Chilli Plants at 3 and 40 Days after the Chilli Plants were Transferred to <i>S. rolfsii</i> -infested Soil in Glasshouse Experiments.....	129
10	Percentages of Surviving Chilli Plants at 20 Days after the Chilli Plants were Transferred to <i>S. rolfsii</i> -infested Soil in Glasshouse Experiments.....	130



Table		Page
11	Populations of the Antagonists in Glasshouse Experiments.....	131
12	Number of <i>S. rolfsii</i> Propagules in Soils around the Chilli Plants at Initially, 1, 2 and 4 Months after Transplanting.....	136
13	Percentages of Surviving Chilli Plants at 2 and 4 Months after Transplanting to <i>S. rolfsii</i> -infested Soil in Small Scale Field Trial.....	137
14	Populations of the Antagonists in a Small Scale Field Trial.....	138
15	Three Comparative Studies on Populations of the Antagonists Incorporated into Amina.....	140
16	Effect of Temperature on Radial Growth of <i>T. harzianum</i> and <i>G. virens</i> on PDA.....	168
17	Effect of pH on Radial Growth of <i>T. harzianum</i> and <i>G. virens</i> on PDA	169
18	Effect of Soil Moisture on Survival and Proliferation of <i>T. harzianum</i> and <i>G. virens</i>	170

LIST OF FIGURES

Figure		Page
1	Measurement of Radial Growth of <i>S. rolfsii</i> in Control and Dual Culture Plates.....	59
2	Diagram showing Paired Culture to Observe Simultaneous Antagonist Effects of <i>T. harzianum</i> and <i>G. virens</i> on <i>S. rolfsii</i>	67
3	Comparison of the Antagonistic Effect of <i>T. harzianum</i> IMI No. 353529 and <i>G. virens</i> IMI No. 353523 on <i>S. rolfsii</i> from Dual Culture and Colony Degradation Test.....	78
4	Radial Growth (A) and Spore Production (B) of <i>T. harzianum</i> on PDA at Different Temperatures.....	91
5	Radial Growth (A) and Spore Production (B) of <i>G. virens</i> on PDA at Different Temperatures.....	93
6	Radial Growth (A) and Spore Production (B) of <i>T. harzianum</i> on PDA Adjusted to Different pH Levels.....	94
7	Radial Growth (A) and Spore Production (B) of <i>G. virens</i> on PDA Adjusted to Different pH Levels.....	95
8	Effect of Soil Moisture on Survival and Proliferation of <i>T. harzianum</i> (A) and <i>G. virens</i> (B).....	97
9	Lay-out of Field Experiment.....	119



LIST OF PLATES

Plate		Page
1	Mycelia and Sclerotia of <i>S. rolfsii</i> on Stem Collar of Infected Chilli Collected from Slim River Estate.....	28
2	Artificial Inoculation of Chilli Stem.....	29
3	Pure Culture of 15-day-old <i>S. rolfsii</i> on PDA (A) and Sclerotia of <i>S. rolfsii</i> from 18-day-old PDA Culture: Mature (Dark Brown) and Young (Creamy Brown and Light Brown) (B).....	33
4	Pre-penetration of <i>S. rolfsii</i> on Chilli Stem Segments: Eruptive Germination of Sclerotium of <i>S. rolfsii</i> 24 hr after Incubation (A) and Ramification of Hyphal Strands on Infected Stem Segments 48 hr after Incubation (B).....	34
5	Disease Development on Stem Segment at 96 hr after Incubation: Severely Damaged Tissue showing Brown, Water-soaked Lesion (A) and Continuously Growing Hyphal Strands on Stem Segment in Direction toward the Shoot (B).....	36
6	Light Microscopy using Interference Contrast System (A) and Scanning Electron Microscopy (B) showing Hyphae of <i>S. rolfsii</i> from Mycelial Aggregates Penetrating into Tissues of Chilli Stem Segment, 48 hr after Incubation.....	37
7	Light Microscopy using Interference Contrast System (A) and Scanning Electron Microscopy (B) showing Hyphae with Enlarge Tips of <i>S. rolfsii</i> Penetrated into Tissues of Chilli Stem Segment, 48 hr after Incubation.....	38
8	Light Microscopy using Interference Contrast System (A) and Scanning Electron Microscopy (B) showing Granulation of Cytoplasm in Cortical Cells Prior to Invasion by Hyphae of <i>S. rolfsii</i>	39
9	Light Microscopy using Interference Contrast System showing Branching and Colonisation of Hyphae of <i>S. rolfsii</i> , 48 hr after Incubation (A) and Constriction of Hyphae during Penetration through Cell Wall of Host Cell (B).....	40



Plate		Page
10	Root Infected by <i>S. rolfsii</i> , 48 hr after Inoculation and Typical Lesion Forming.....	42
11	72 hr after Inoculation: Colonisation of Chilli Stem by <i>S. rolfsii</i> (A) and Colonisation and Ramification on Stem, both Underground and Above-ground Parts (B).....	43
12	Stem Base showing Brown, Water-soaked Lesion with the Production of Sclerotia of <i>S. rolfsii</i> on the Soil Surface, 120 hr after Inoculation.....	44
13	Light Microscopy using Interference Contrast System showing Mycelial Aggregates found 72 hr after Inoculation appearing as Masses of Hyphae (A) or Dome-shaped Structure (B).....	46
14	Scanning Electron Microscopy showing Mycelial Aggregates producing Penetration Hyphae to Penetrate into the Host Cells, at 72 hr after Incubation.....	47
15	Light Microscopy using Interference Contrast System (A) and Scanning Electron Microscopy (B) showing Hyphae with Enlarge Tips Produced from Mycelial Aggregate Exerting Physical Pressure on the Epidermal Layer.....	48
16	Colony Appearance of 5-day-old Pure Culture of Nine Isolates of <i>T. harzianum</i> on PDA.....	69
17	Light Microscopy using Interference Contrast System of Nine Isolates of <i>T. harzianum</i>	70
18	<i>G. virens</i> (IMI No. 353523): 5-day-old Pure Culture on PDA (A) and Light Microscopy using Interference Contrast System of Conidia and Conidiophores (B).....	71
19	Antagonistic Effect of <i>T. harzianum</i> and <i>G. virens</i> Isolates to <i>S. rolfsii</i> in Dual Culture.....	74
20	Antagonistic Effect of <i>T. harzianum</i> and <i>G. virens</i> Isolates on <i>S. rolfsii</i> , as Indicated by the Clear Zones, in the Colony Degradation Test.....	77

Plate		Page
21	Light Microscopy using Interference Contrast System showing <i>T. harzianum</i> Produced Hooks (A) Attaching on and Short Branch (B) Penetrating into <i>S. rolfsii</i> Hypha.....	80
22	Light Microscopy using Interference Contrast System showing Hyphae of <i>T. harzianum</i> Produced Short Branches to Penetrated into <i>S. rolfsii</i> Hypha.....	81
23	SEM Micrographs showing Hyphae of <i>G. virens</i> Coiling around (A) or Hooking on (B) Hyphae of <i>S. rolfsii</i>	82
24	Light Microscopy using Interference Contrast System showing Hyphae of <i>G. virens</i> Compactly Coiled around Hyphae of <i>S. rolfsii</i>	83
25	SEM Micrographs of Hyphae of <i>T. harzianum</i> (A) and <i>G. virens</i> (B) Penetrating through Cuticle of Sclerotium of <i>S. rolfsii</i> , at 24 hr after Incubation.....	85
26	SEM Micrographs of Cross-section of Healthy Sclerotia of <i>S. rolfsii</i> Taken from the 1-month-old PDA Culture (A) and Damaged Medulla of Sclerotium of <i>S. rolfsii</i> Parasitised by <i>T. harzianum</i> at 12 Days after Exposing to the Antagonist (B).....	86
27	SEM Micrographs of Damaged Medulla of Sclerotium of <i>S. rolfsii</i> Parasitised by <i>G. virens</i> (A) and Chlamydospores of <i>G. virens</i> Found among Damaged Hyphae of Medulla (B).....	87
28	Dual Culture of <i>T. harzianum</i> and <i>G. virens</i> on PDA for Inhibition Observation at 2 and 4 Days after Incubation.....	99
29	Paired Cultures of <i>T. harzianum</i> vs <i>S. rolfsii</i> and <i>G. virens</i> vs <i>S. rolfsii</i> on PDA at 4 and 7 Days after Incubation.....	100
30	<i>T. harzianum</i> and <i>G. virens</i> Recovered on <i>Trichoderma</i> Medium E.....	123

Plate		Page
31	Abundant Sclerotia of <i>S. rolfsii</i> Found on Surface of the <i>S. rolfsii</i> -infested Soil in Plastic Tray of the Control Treatment (A) and the Parasitised sclerotia by <i>G. virens</i> at 3 Days after Transplanting the Chilli Plants to <i>S. rolfsii</i> -infested Soil (B).....	127
32	Evaluation of Viable <i>S. rolfsii</i> Propagules in Soil Samples Taken from the Area around the Chilli Plants at 3 Days after Transplanting.....	128

Abstract of dissertation submitted to the Senate of
Universiti Pertanian Malaysia in fulfilment of the requirements for
the degree of Doctor of Philosophy.

EVALUATION OF MALAYSIAN ISOLATES OF *TRICHODERMA HARZIANUM*
RIFAI AND *GLIOCLADIUM VIRENS* MILLER, GIDDENS AND FOSTER
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OF CHILLI

By

JINANTANA JOMDUANG

April 1995

Chairman : Assoc. Prof. Dr. Sariah Meon
Faculty : Agriculture

Sclerotium foot rot of chilli (*Capsicum annuum* L.) caused by *Sclerotium rolfsii* Sacc. is commonly found to cause damage in almost all the chilli growing areas in Malaysia. Conventional methods to control the disease, for example, fungicide drenching and crop rotation, seem to be uneconomical and impractical. Recent years, integrated pest management strategy is being adopted for plant disease control which reduces or have lesser impact on the environment. The present study was carried out to evaluate the Malaysian isolates of *Trichoderma harzianum* Rifai and *Gliocladium virens* Miller, Giddens and Foster as biocontrol agents for Sclerotium foot rot of chilli both in glasshouse and small scale field trials. Infection mechanism of *S. rolfsii* on chilli plants and the antagonistic effects of *T. harzianum* and *G. virens* on *S. rolfsii* were also studied.



S. rolfsii was isolated from naturally infected chilli plants collected from Slim River Estate, Perak, Malaysia. Infection mechanism of *S. rolfsii* was observed on both stem segments and the 2-month-old chilli plants, local variety "Langkap". Mycelial aggregates, formed on stem surface along with the ramifying hyphal strands, showed a distinct role in facilitating penetration of *S. rolfsii* into the host cells. Success in penetration through the epidermis was due to mechanical pressure induced by penetration hyphae produced from mycelial aggregates, and cell wall degrading enzyme activity. There was no specific site on the chilli stem for initiation of mycelial aggregates and majority of them appeared as flat masses of hyphae. Few were dome-shaped structures.

The target sites of infection by *S. rolfsii* were found to be the stem collar and underground stem although primary ingression could either be on the stem collar or roots depending on the placement of the inoculum. Further, the 2-month-old chilli plants had shown to be more susceptible to *S. rolfsii* than the 4-month-old ones. However, high concentration of *S. rolfsii* propagules was needed to cause foot rot disease in the older plants.

Nine isolates of *T. harzianum* and one isolate of *G. virens* were isolated from soils and *S. rolfsii* mycelia found on infected chilli plants, respectively, collected from Slim River and Trolak Estates, Perak, Malaysia. Screening for potential *T. harzianum* and *G. virens* antagonistic as biocontrol agents to *S. rolfsii* was

carried out using dual culture and colony degradation test. *T. harzianum* IMI No. 353529 and *G. virens* IMI No. 353523 were the most effective isolates and were used throughout further studies. Antagonism mechanism of these two isolates on *S. rolfii* was observed under light and scanning electron microscopes. *T. harzianum* and *G. virens* were capable of parasitising both hyphae and sclerotia of *S. rolfii*. These two antagonistic isolates also produced volatile and non-volatile inhibitors that could retard radial growth of *S. rolfii* on potato dextrose agar (PDA). Age of antagonists was found to have a significant effect ($p < .01$) on their ability to produce both volatile and non-volatile inhibitors.

Predisposing factors affecting growth and proliferation of *T. harzianum* and *G. virens* were studied. *T. harzianum* grew and proliferated well over a temperature range of 20–30°C, a pH range of 3.8–5.8 and a soil moisture content of 10–25%. *G. virens* was found to grow and proliferate well over the same temperature range, the pH range of 3.8–7.2 and the same soil moisture content.

Inhibition between *T. harzianum* and *G. virens* was not observed on plate culture. In addition, the antagonistic effect of the two antagonists against *S. rolfii* on paired culture assay could occur simultaneously.

Shelf-life of antagonists (*T. harzianum*, *G. virens* and *T. harzianum* + *G. virens*) incorporated to three types of commercially available organic fertilizer (Amina, Green Supergro and Avanti Green) kept at room temperature were evaluated. It was found

that all antagonists incorporated to Amina and Avanti Green had a significantly ($p < .01$) higher shelf-life period when compared to those incorporated to Green Supergro.

Glasshouse trial to evaluate the efficacy of the antagonists (*T. harzianum*, *G. virens*, and *T. harzianum* + *G. virens*) either alone or incorporated to three types of organic fertilizer (Amina, Green Supergro and Avanti Green) in controlling Sclerotium foot rot of chilli, local variety "Kulai", was carried out. The result showed that soil amended with antagonists and Amina had the lowest number of viable *S. rolfsii* propagules per 100 g air-dried soil. In addition, populations of the antagonists in soils around the chilli plants (within 5 cm radius from stem and 10 cm depth) and on roots were found to be higher and subsequently the percentages of surviving plants.

Small scale field trial was carried out to evaluate the efficacy of antagonists (*T. harzianum*, *G. virens*, and *T. harzianum* + *G. virens*) either alone or incorporated to Amina, which was selected based on its effectiveness from the shelf-life study and glasshouse trial, compared to PCNB in controlling Sclerotium foot rot of chilli. The result showed that *G. virens* incorporated to Amina was superior to all other treatments. This antagonist preparation effectively decreased *S. rolfsii* propagules in soils around the chilli plants (within 5 cm radius from stem and 10 cm depth). It also gave higher percentage of surviving plants and number of antagonist population in soils around the chilli plants.

Abstrak disertasi yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk ijazah Doktor Falsafah

PENILAIAN PENCILAN-PENCILAN *TRICHODERMA HARZIANUM* RIFAI
DAN *GLIOCLADIUM VIRENS* MILLER, GIDDENS DAN FOSTER DARI
MALAYSIA UNTUK KAWALAN BIOLOGI REPUT PANGKAL
SCLEROTIUM CILI

oleh

JINANTANA JOMDUANG

April 1995

Pengerusi : Prof. Madya Dr. Sariah Meon
Fakulti : Pertanian

Reput pangkal cili (*Capsicum annuum* L.) yang disebabkan oleh *Sclerotium rolfsii* Sacc. didapati menyebabkan kerosakan di kebanyakan kawasan penanaman cili di Malaysia. Kaedah pengawalan penyakit tradisional seperti penyiraman racun kulat dan penggiliran tanaman, menunjukkan tidak ekonomik dan praktik. Kebelakangan ini strategi pengurusan perosak bersepadu telah diamalkan untuk pengawalan penyakit tanaman di mana ia kurang memberi kesan terhadap persekitaran. Kajian berikut dijalankan untuk menilai pencilan-pencilan *Trichoderma harzianum* Rifai dan *Gliocladium virens* Miller, Giddens dan Foster dari Malaysia sebagai agen kawalan biologi untuk reput pangkal *S. rolfsii* pada tanaman cili di dalam rumah kaca dan percubaan-percubaan ladang secara kecil-kecilan. Mekanisme jangkitan *S. rolfsii* ke atas pokok cili dan kesan antagonis *T. harzianum* dan *G. virens* ke atas *S. rolfsii* juga dikaji.

S. rolfsii telah dipencilkan daripada tanaman cili yang dijangkiti secara semula jadi dari Slim River Estate, Perak, Malaysia. Mekanisme jangkitan *S. rolfsii* diperhatikan pada kedua-dua segmen batang dan pokok cili, varieti tempatan "Langkap", berumur dua bulan. Agregat miselium didapati terbentuk di atas permukaan batang bersama-sama dengan helaian hifa yang bercabang-cabang. Struktur ini menunjukkan peranan khusus dalam memudahkan penembusan *S. rolfsii* ke dalam sel-sel perumah. Kejayaan dalam penembusan melalui epidermis adalah disebabkan oleh tekanan mekanikal yang diaruh oleh hifa penembusan yang terhasil daripada agregat-agregat miselium dan aktiviti enzim penghancur dinding sel. Penembusan seterusnya ke dalam sel-sel perumah berlaku secara inter- dan intra-sel. Tidak terdapat tapak yang khusus pada batang cili untuk inisiasi agregat miselium dan kebanyakan daripada mereka kelihatan sebagai massa hifa yang rata. Ada juga yang kelihatan sebagai struktur berbentuk kubah.

Tapak sasaran jangkitan oleh *S. rolfsii* telah didapati pada pangkal batang dan bahagian batang bawah tanah, walaupun penembusan prima mungkin boleh berlaku pada pangkal batang atau akar bergantung kepada penempatan inokulum. Tanaman cili yang berumur dua bulan telah menunjukkan kerentanan yang lebih kepada *S. rolfsii* daripada tanaman yang berumur empat bulan. Walau bagaimanapun, kepekatan propagul *S. rolfsii* yang tinggi diperlukan untuk menyebabkan penyakit reput pangkal pada pokok-pokok cili yang lebih tua.

Sembilan pencilan *T. harzianum* dan satu pencilan *G. virens* yang telah diasingkan daripada tanah dan miselium *S. rolfsii* yang didapati pada tanaman cili yang berpenyakit, masing-masing telah dikutip daripada Estet Slim River dan Estet Trolak, Perak, Malaysia. Penyaringan untuk ciri antagonis *T. harzianum* dan *G. virens* yang berpotensi sebagai agen kawalan biologi terhadap *S. rolfsii* dijalankan dengan menggunakan kultur kembar dan ujian degradasi koloni. *T. harzianum* IMI No. 353529 dan *G. virens* IMI No. 353523 merupakan pencilan yang paling berkesan dan akan digunakan untuk kajian-kajian seterusnya. Mekanisme antagonisme kedua-dua pencilan terhadap *S. rolfsii* diperhatikan di bawah mikroskop cahaya dan mikroskop elektron "scanning". *T. harzianum* dan *G. virens* berupaya memparasit kedua-dua hifa dan sklerotia *S. rolfsii*. Kedua-dua pencilan antagonis ini juga mengeluarkan perencat meruap dan tak meruap yang boleh merencat pertumbuhan radial *S. rolfsii* di atas PDA. Umur antagonis didapati mempunyai kesan bererti ($p < .01$) ke atas keupayaannya untuk mengeluarkan perencat meruap dan tak meruap.

Faktor-faktor pradedah yang mempengaruhi pertumbuhan dan proliferasi *T. harzianum* telah dikaji. *T. harzianum* hidup dan proliferasi dengan baik pada julat suhu 20–30°C, julat pH 3.8–5.8 dan kandungan kelembapan tanah 10–25%. *G. virens* didapati hidup dan proliferasi dengan baik juga pada julat suhu yang sama, julat pH 3.8–7.2 dan kandungan kelembapan tanah yang sama.

Ujikaji di ladang dijalankan untuk menilai keberkesanan antagonis-antagonis (*T. harzianum*, *G. virens* dan *T. harzianum* + *G. virens*) sama ada sendirian atau digabungkan dengan Amina, yang dipilih berdasarkan kepada keberkesanannya daripada kajian jangka hayat rak dan rumah kaca, dibandingkan dengan PCNB untuk pengawalan reput pangkal *Sclerotium* tanaman cili. Keputusan menunjukkan bahawa *G. virens* yang digabungkan dengan Amina adalah yang terbaik sekali di antara kesemua rawatan. Penyediaan antagonis berupaya menurunkan bilangan propagul *S. rolfsii* dalam tanah di sekitar pokok cili (di sekitar jejari 5 cm daripada batang dan kedalaman 10 cm). Ia juga memberi peratusan yang tinggi bagi pokok yang hidup dan populasi antagonis yang tinggi dalam tanah di sekitar pokok cili.

CHAPTER I

INTRODUCTION

Chilli has been an important food crop for an extensive period of time. It is native to tropical America and was grown in North and South America for over 2,000 years ago (Splittstoesser, 1979). Chilli belongs to the genus *Capsicum* of the Solanaceae family. Two popular cultivated species are *Capsicum annuum* L. and *Capsicum frutescens* L. The first species constitutes a large number of cultivated varieties and is by far the most important species (Salunkhe and Desai, 1984). The pungency in chilli is dependant on the amount of a substance called capsaicin which is found in high concentration in the placenta of the fruit. Accordingly, chilli can be divided into two groups: i) those producing hot or pungent fruits, and ii) those bearing mild or sweet fruits.

Choudhury (1984) stated that chilli is a good source of vitamins A and C. These two vitamin constituents amount to 1,000 IU and 110 mg per 100 g fresh weight, respectively, which are evidently higher than those found in tomato, snap bean, yellow vegetables, whole wheat and rice.

Chilli is one of the main fruit vegetables in Malaysia. It is grown in an area of approximately 1,153 hectares with an annual production of about 17,237 tonnes (Syed et al., 1992). It is also